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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/593,914	06/14/2000	Jens J. Hyldig-Nielsen	BP9901US	8319

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EXAMINER	
MYERS, CARLA J	
ART UNIT	**PAPER NUMBER**
1634	

DATE MAILED: 10/20/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/593,914	HYLDIG-NIELSEN ET AL.	
	Examiner	Art Unit	
	Carla Myers	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *25 July 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *10-12,16,18,19,21-26,29,32,34,46-49,61,62 and 80-104* is/are pending in the application.

4a) Of the above claim(s) *34 and 99-104* is/are withdrawn from consideration.

5)☒ Claim(s) *46-49,61,62,83-85 and 88-98* is/are allowed.

6)☒ Claim(s) *10-12,16,18,19,21-26,29,32,80-82,86 and 87* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. This action is in response to the amendment filed July 25, 2005. Applicant's arguments have been fully considered but are not persuasive to overcome all grounds of rejection. All rejections not reiterated herein are hereby withdrawn. This action is made final.

2. With respect to claims 86 and 87, the phrase "previously new" is not an acceptable status identifier. Claims 86 and 87 should be accompanied by the status identifier of "previously presented." See 37 CRF 1.21 (c) for a listing of acceptable status identifiers.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Claims 80-82 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kosse in view of Stender (1998) and further in view of Parton (U.S. Patent No. 5,905,038).

Kosse discloses dot blot and *in situ* hybridization methods for the detection and enumeration of *Dekkera bruxellensis*. Kosse teaches that the *in situ* hybridization method is performed using fluorescent labeled probes (e.g., TRITC or FLUOS) and that the dot blot hybridization is performed using digoxigenin labeled probes (page 469). The probes are complementary to sequences of the *Dekkera bruxellensis* 18S rRNA region (page 469; Table 2). The reference teaches that prior to *in situ* hybridization, yeast cell walls must be permeabilized and that probes must be selected to yeast 18S rRNA regions which are fully accessible to probes (see page 478). Kosse teaches that *Dekkera bruxellensis* was successfully detected by in situ hybridization using 20% formaldehyde (see Table 2 and page 474). Table 2 lists additional 18S rRNA probes for the detection of other yeasts known to cause spoilage of dairy products (see page 468 and Table 2). Probes are also disclosed which are specific for all yeasts and for all eukaryotes (Table 2). Kosse further teaches that it is essential to provide accurate methods for detecting the presence of yeast in dairy products and other foods so as to ensure high quality and safe food products (see page 468).

Kosse teaches that the *in situ* hybridization method is performed using fluorescent- labeled probes and that dot blot hybridization is performed using digoxigenin labeled probes. Kosse does not specifically teach using enzyme-linked probes, or specifically soy bean peroxidase labeled probes.

However, Stender (1998) teaches that enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of Kosse so as to have specifically labeled the probes with soy bean peroxidase in order to have achieved the benefit of providing an effective means for labeling the probes, thereby facilitating the detection of *Dekkera bruxellensis*.

Additionally, the combined references do no teach filtering the microorganism and culturing the microorganism on a filter prior to performing in situ hybridization.

However, Parton (col. 1-2) teaches methods for isolating and analyzing samples for the presence of microorganisms wherein the methods comprise filtering a sample containing microorganisms through a membrane filter so as to trap the microorganisms on the filter and then culturing the microorganisms on the filter.

In view of the teachings of Parton, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kosse in view of Stender so as to have isolated the microorganisms by filtration and then cultured the microorganisms on the filter prior to performing in situ hybridization in order to have provided an effective means for purifying and concentrating the microorganisms and for increasing the number of microorganisms to allow for the formation of colony forming units so as to increase the accuracy and sensitivity of the detection method.

Furthermore, Kosse and Stender do not teach kits comprising filters and culture media. However, the method of Kosse in view of Stender requires the use of fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, and film. In view of the teachings of Parton, modification of the method of Kosse and Stender to include a filtration and culturing step would have resulted in a method which further required the use of a filter and culture media. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, film, filter and culture media in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis* by in situ hybridization and dot blot hybridization.

With respect to claim 82, Kosse does not teach using PNA probes. However, Stender (see, for example, pages 3 and 10-11) teaches PNA probes complementary to rRNA sequences which are useful for the detection of microorganisms. Stender teaches that PNA probes hybridize to RNA or DNA with a higher affinity and specificity than their nucleic acid counterparts. PNA probes are also more stable due to their resistance to naturally occurring nucleases and proteases. Methods are disclosed for modifying nucleic acid probes so as to incorporate peptide nucleic acid moieties (see, for example, pages 13-14). Stender also teaches that PNA probes can be used in either *in situ* or *in vitro* hybridization methods (page 23). In view of the teachings of Stender, it would have been obvious to one of ordinary skill in the art at the time the

invention was made to have modified the probes of Kosse by including peptide nucleic acid moieties in the probes and thereby generating PNA probes, in order to have provided probes with increased affinity and specificity and increased resistance to nucleases and proteases.

Response to arguments:

In the response, Applicants traverse this rejection. Applicants arguments are directed to the unobviousness of using an enzyme labeled probe to detect yeast by in situ hybridization. However, the present claims are not limited to such methods. Rather, the present claims are drawn to kits. The combined references teach the reagents required to detect *Dekkera bruxellensis* by dot blot and in situ hybridization, including the reagents of fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, film, filter and culture media. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have packaged these reagents in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis* by in situ hybridization and dot blot hybridization.

Applicants response further states that the rejection does not provide the motivation for combining the references. However, it is maintained that Stender provides the motivation for labeling the probes with an enzyme label. Stender teaches that nucleic acid probes may be effectively labeled with enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). Thereby,

Stender provides the motivation to label the *Dekkera bruxellensis* dot blot probe with an enzyme label because this would have provided an effective means for facilitating the detection of complexes formed between the probe and target nucleic acid. Further, Parton provides the motivation for filtering the microorganism and culturing the microorganism on a filter prior to performing in situ hybridization. In particular, Parton (col. 1-2) teaches that by filtering a sample through a filter, microorganisms present in the sample are trapped on the filter and can be grown directly on the filter prior to their analysis. The rejection does in fact state the motivation for combining the teachings of Paron with Kosse in Stender and specifically states that isolating the microorganisms by filtration and culturing the microorganisms on the filter prior to performing in situ hybridization provides the advantage of purifying and concentrating the microorganisms and for increasing the number of microorganisms to allow for the formation of colony forming units so as to increase the accuracy and sensitivity of the detection method.

4. Claims 10-12, 16, 18, 19, 21-26, 29, 32, 86 and 87 are rejected under 35 U.S.C. 103(a) as being unpatentable over De Wachter in view of Kosse and further in view of Stender et al (1998; reference BB).

De Wachter teaches an isolated nucleic acid consisting of the sequence of the 18S rRNA of *Dekkera/Brettanomyces bruxellensis*. The 18S rRNA of De Wachter comprises the sequence of SEQ ID NO: 1 (see nucleotides 1066-1052 of GenBank Accession No. X58052). The nucleic acid of De Wachter is considered to have the property of being suitable as a probe for the detection, identification or quantitation of *Dekkera/Brettanomyces bruxellensis*. De Wachter does not teach labeling the 18S

rRNA with a detectable moiety, and particularly does not teach labeling the probe with an enzyme.

Kosse teaches hybridization methods, including dot blot hybridization and in situ hybridization, for the detection of *Dekkera bruxellensis*. Kosse teaches labeling probes with either chemiluminescent labels (e.g., digoxigenin) or fluorescent labels (e.g., TRITC or FLUOS) to facilitate the detection of yeasts and to particularly facilitate the detection of *Dekkera bruxellensis* (see page 469). The reference further exemplifies an 18S rRNA probe specific for *Dekkera bruxellensis* (see Table 2) and probes specific for other yogurt spoiling yeasts. Kosse teaches that it is essential to provide accurate methods for detecting the presence of yeast in dairy products, such as yoghurt, so as to ensure high quality and safe food products (see page 468).

In view of the teachings of Kosse, it would have been further obvious to one of ordinary skill in the art at the time the invention was made to have used the labeled 18S rRNA of De Wachter as a probe under suitable hybridization conditions in order to have facilitated the detection of *Dekkera bruxellensis* in dairy samples. Additionally, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have generated probe sets comprising one or more probes for *Dekkera bruxellensis* or comprising probes for *Dekkera bruxellensis* and probes for other yeast and to have labeled each probe with a different detectable moiety in order to have allowed for the detection and differentiation of multiple types of yeast in dairy products, such as yoghurt.

The combined teachings of De Wachter and Kosse do not teach labeling the probes with soy bean peroxidase. However, Stender teaches that enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of De Wachter in view of Kosse by using soy bean peroxidase to label the probes in order to have provided an equally effective probe for the detection of *Dekkera bruxellensis.*

Secondly, De Wachter and Kosse do not teach immobilizing the *D. bruxellensis* and/or yeast probes onto a solid support. However, Stender teaches that hybridization probes may be immobilized onto solid supports and particularly may be in the format of an array (page 31). It is stated that the use of an array provides the advantage of allowing for the simultaneous analysis using 100 or more different probes. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have immobilized the probes of De Wachter and Kosse onto a solid support, as taught by Stender, in order to have achieved the benefit of simultaneously assaying for the presence of target sequences complementary to a multitude of different probes.

Thirdly, the combined references do not teach PNA probes for the detection of *Dekkera bruxellensis*. However, Stender (see, for example, pages 3 and 10-11) teaches PNA probes complementary to rRNA sequences which are useful for the detection of microorganisms. Stender teaches that PNA probes hybridize to RNA or

DNA with a higher affinity and specificity than their nucleic acid counterparts. PNA probes are also more stable due to their resistance to naturally occurring nucleases and proteases. Methods are disclosed for modifying nucleic acid probes so as to incorporate peptide nucleic acid moieties (see, for example, pages 13-14). Stender also teaches that PNA probes can be used in either *in situ* or *in vitro* hybridization methods (page 23). In view of the teachings of Stender, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of De Wachter and Kosse by including peptide nucleic acid moieties in the probes and thereby generating PNA probes, in order to have provided probes with increased affinity and specificity and increased resistance to nucleases and proteases.

With respect to claim 25, De Wachter and Kosse do not teach adding blocking probes to the probe sets. Stender (page 25, 28) teaches adding blocking probes (i.e., random non-selected probes) to hybridization reactions in order to reduce non-specific binding. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe sets of De Wachter and Kosse so as to have included the "blocking probes" disclosed by Stender in order to have accomplished the objective of reducing non-specific binding of the yeast probes.

Response to arguments:

In the response, Applicants traverse this rejection by stating that Kosse teaches that only the 3' end of the 18S rRNA is accessible to fluorescently labeled probes and that other variable regions are not accessible to in-situ hybridization. Applicants state that "it is not reasonable for the Examiner to baldly assert that any nucleobase

sequence that is homologous to the gene sequence described by De Wachter will be useful to produce an in situ hybridization probe." This argument has been fully considered but is not persuasive because Applicants arguments are not directed to limitations recited in the claims since the present claims are not drawn to in situ hybridization probes.

Applicants point to teachings of Kosse in the abstract at on page 474. Each of the cited teachings is directed only to in situ hybridization. In particular, Applicants cited Kosse as teaching "Whole cell hybridization experiments revealed that the 3' end of the target molecule is a suitable site for fluorescently labeled species specific probes...Other variable regions of the 18S rRNA tested for species specific probes were not accessible to in situ hybridization." Applicants further cite page 474 of Kosse as teaching "*In situ* identification of whole cells was performed with 11 species-specific, two group-specific and the universal probe EUK516...Experiments with a variety of species-specific fluorescently labeled oligonucleotides revealed that only the 3'-end of the 18S rRNA is accessible for species-specific probes." Accordingly, these teachings of Kosse are limited to species specific hybridization probes used in in situ hybridization. However, the present claims are not limited to this subject matter.

Applicants state that "The contradiction between the Examiner's assertions and the actual teachings of Kosse is self-evident. That the Examiner has relied upon a misinterpretation of the teachings of a reference in formulating the present rejection can lead to only one logical conclusion. That the rejection is based upon incorrect factual predicates and therefore is improper." This argument has been fully considered but is

not persuasive. The statements set forth in the rejection are not contrary to the teachings of Kosse. Applicants arguments are based only on the teachings of Kosse as they relate to in situ hybridization probes. However, Kosse also teaches dot blot hybridization probes and the statements regarding the 3'-end of the rRNA are not directed to the dot blot hybridization probes. Kosse does not disclose any secondary structure present in the 3'-end of the rRNA probes that would interfere in dot blot hybridization. Accordingly, again, Applicants arguments are directed only to in situ hybridization probes. However, the present claims are drawn broadly to include dot blot hybridization probes for detecting Dekkera yeasts.

Applicants response further states that the rejection does not provide the motivation for combining the references. However, it is maintained that Stender provides the motivation for labeling the probes with an enzyme label. Stender teaches that nucleic acid probes may be effectively labeled with enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). Thereby, Stender provides the motivation to label the *Dekkera bruxellensis* dot blot probe with an enzyme label because this would have provided an effective means for facilitating the detection of complexes formed between the probe and target nucleic acid. With respect to claims 12 and 26, as set forth in the rejection, Stender teaches that PNA probes hybridize to RNA or DNA with a higher affinity and specificity than their nucleic acid counterparts. PNA probes are also more stable due to their resistance to naturally occurring nucleases and proteases. Thereby, Stender provides the motivation to use

PNA hybridization probes to achieve the advantage of increasing the increased affinity and specificity of the probes, as well as increasing the resistance of the probes to nucleases and proteases. With respect to claims 18 and 32, as set forth in the rejection, Stender provides the motivation to immobilize the probes onto a solid in order to have achieved the benefit of simultaneously assaying for the presence of target sequences complementary to a multitude of different probes. With respect to claim 25, as set forth in the rejection, Stender also provides the motivation to include blocking probes with the hybridization probes in order to achieve the advantage of reducing non-specific binding of the yeast probes.

5. Claims 80-82 are rejected under 35 U.S.C. 103(a) as being unpatentable over De Wachter in view of Kosse and Stender (1998) and further in view of Parton (U.S. Patent No. 5,905,038).

The teachings of De Wachter, Kosse and Stender are presented above. The combined references do no teach filtering the microorganism and culturing the microorganism on a filter prior to performing in situ hybridization.

Parton (col. 1-2) teaches methods for isolating and analyzing samples for the presence of microorganisms wherein the methods comprise filtering a sample containing microorganisms through a membrane filter so as to trap the microorganisms on the filter and then culturing the microorganisms on the filter.

In view of the teachings of Parton, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kosse in view of Stender so as to have isolated the microorganisms by filtration and then

cultured the microorganisms on the filter prior to performing in situ hybridization in order to have provided an effective means for purifying and concentrating the microorganisms and for increasing the number of microorganisms to allow for the formation of colony forming units so as to increase the accuracy and sensitivity of the detection method.

Furthermore, the combined references do not teach kits comprising filters and culture media. However, the method of Kosse in view of Stender requires the use of fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, and film. In view of the teachings of Parton, modification of the method of De Wachter, Kosse and Stender to include a filtration and culturing step would have resulted in a method which further required the use of a filter and culture media. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, film, filter and culture media in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis* by in situ hybridization and dot blot hybridization.

Response to arguments:

In the response, Applicants traverse this rejection for the same reasons stated in above. Accordingly, the response to those arguments applies equally to the present grounds of rejection.

6. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Schut (WO 99/10533) discusses methods of in situ hybridization to detect the presence of a microorganism wherein the methods comprise treating a microorganism with a solution to partly degrade the cell wall or membrane to thereby allow for penetration through the cell wall or membrane of an enzyme labeled probe (see abstract, claim 1, and pages 6-7). Schut teaches that probes labeled with horseradish peroxidase are too large to freely enter bacterial cells and therefore methods are needed to permeabilize the cell wall (page 16). Schut (page 4) notes that permeabilization of cells "gets more difficult for larger molecules since the margin between the accessibility of the target molecules and loss of target molecules or complete cell lysis becomes very narrow." To overcome these obstacles, Schut discloses specific conditions and reagents that can be used to permeabilize bacteria (see Table 8 and Example 9). The reference also discloses methods for linking probes to HP in order to provide a "probe molecule which is small enough to penetrate a cell wall when this barrier is properly and controllably degraded."

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of

the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carla Myers whose telephone number is (571) 272-0747. The examiner can normally be reached on Monday-Thursday from 6:30 AM-5:00 PM. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (571)-272-0745.

The fax phone number for the organization where this application or proceeding is assigned is (571)-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at (866)-217-9197 (toll-free).

Carla Myers
October 12, 2005

Carla Myers
CARLA J. MYERS
PRIMARY EXAMINER